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## Original research article

# Analgesic effects of antidepressants alone and after their local co-administration with morphine in a rat model of neuropathic pain



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## ABSTRACT

**Background:** The therapy of neuropathic pain may include the use of co-analgesics, such as antidepressants, however, their desired analgesic effect is associated with significant side effects. An alternative approach to this is their local administration which has been proposed, but there is little data regarding their local co-administration with morphine and the nature of the interaction between morphine and either doxepin or venlafaxine, two antidepressant drugs that have been recently used in neuropathic pain therapies.

**Methods:** This study was performed on rats after chronic constriction injury (CCI) to the sciatic nerve. The von Frey and Hargreaves' tests were used to assess mechanical allodynia and thermal hyperalgesia, respectively, after intraplantar (*ipl*) or subcutaneous (*sc*) administration of amitriptyline, doxepin, or venlafaxine, or their *ipl* co-administration with morphine on day 12–16 after injury.

**Results:** The *ipl* administration of amitriptyline (3, 15 mg), doxepin (1, 5, 10, 15 mg), or venlafaxine (2, 7 mg) was effective in antagonizing CCI-induced allodynia. Their *sc* injection at a site distal to the injured side, did not induce alterations in pain thresholds, which supports the local mode of action. Of the three antidepressants used in this study, only *ipl* co-administration of amitriptyline with morphine significantly enhanced its effect in contrast to doxepin and venlafaxine, both of which weakened the analgesic effect of morphine.

**Conclusions:** In summary, the results suggest that when amitriptyline (but not doxepin or venlafaxine) is locally co-administered with morphine the effectiveness under neuropathic pain is enhanced, although additional studies are necessary to explain differential mechanisms of interaction of antidepressant drugs with morphine after local administration.

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## Introduction

Neuropathic pain is defined as pain arising as a direct consequence of a lesion or disease affecting the somatosensory system [1]. The most frequent etiologic factors of neuropathic pain are trauma (including post-surgery scars), metabolic disturbances (e.g., diabetes mellitus) and ischemia. The pathological mechanism of neuropathic pain differs significantly from that of inflammatory

pain; studies have shown that the changes in spinal gene expression under neuropathy and inflammations are different [2]. Thus, the response to antinociceptive drugs, especially opioids, is not the same. It has generally been accepted that neuropathic pain is somewhat resistant to morphine administration in clinical studies [3,4], and the reduced ability of morphine to attenuate allodynia and hyperalgesia in experimental models of neuropathic pain has been demonstrated [5–8]. For these reasons, it is a common clinical practice to use analgesic drug combinations. The primary approach to treat neuropathic pain is the use of coanalgesics such as tricyclic antidepressants (TCA); however, their systemic application is associated with significant side effects, which can hinder the desired analgesic effect. An alternative approach to this is the topical administration of analgesics. Currently, the possibility of the topical

**Abbreviations:** CCI, chronic constriction injury; MOR, morphine; AMT, amitriptyline; DOX, doxepin; VFX, venlafaxine; *ipl*, intraplantar; *sc*, subcutaneous.

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use of these drugs that have local analgesic effects has been proposed [9–14], but there is little data regarding antidepressant topical coadministration with morphine and the nature of the interaction between morphine and either doxepin or venlafaxine, two antidepressant drugs that have been recently used in neuropathic pain therapies.

Tricyclic antidepressants are the most studied group of antidepressants for the treatment of neuropathic pain. These drugs diminish the transmission of nociceptive information from the site of injury by inhibiting the reuptake of serotonin and norepinephrine at the synapse, which leads to prolonged activation of the antinociceptive descending pathways. Interestingly, pain relief appears to be independent of the primary antidepressant effects of these drugs. The effects of coadministration of opioids and antidepressants in different chronic pain models were shown by some authors. The synergistic action of morphine with amitriptyline or doxepine in preclinical and clinical study was demonstrated by some groups [15–18]. Wrzosek et al. [19] has shown that the combined administration of tramadol with doxepin was more effective than tramadol and venlafaxine, which provides valuable information regarding clinical practices and rationalizing the administration of different drug combinations. It should be noted that venlafaxine is a structurally novel antidepressant that inhibits reuptake of 5-hydroxytryptamine and noradrenaline, but unlike older generation of antidepressants, it has few side effects and has become increasingly popular in the treatment of pain [20,21]. Considering the possible local analgesic effects of antidepressants, which may be crucial when they are used in combination with morphine, we evaluated whether the local use of antidepressants (amitriptyline, doxepin and venlafaxine) is effective in the chronic constriction injury model of neuropathic pain in rats and if local coadministration of these antidepressants with morphine could influence its analgesic effect under neuropathic pain conditions.

## Materials and methods

### Animals

Male Wistar rats (250–350 g) were obtained from Charles River (Hamburg, Germany) and housed in cages lined with sawdust under a standard 12/12 h light/dark cycle (lights on at 08:00 h) with food and water provided ad libitum. All experiments were performed according to the recommendations of IASP [22] and the NIH Guide for the Care and Use of Laboratory Animals and were approved by the local Bioethics Committee (Kraków, Poland).

### Surgical preparations

Chronic constriction injury (CCI) was generated as previously described by Bennett and Xie [23]. Four ligatures were tied around the sciatic nerve under sodium pentobarbital anesthesia (60 mg/kg; *ip*). The biceps femoris and the gluteus superficialis were separated, and the right sciatic nerve was exposed. The ligatures (4/0 silk) were tied loosely around the nerve distal to the sciatic notch at 1 mm distances until they elicited a brief twitch in the respective hind limb. After surgery, all of the animals (100%) developed long-lasting neuropathic pain symptoms, including tactile allodynia and thermal hyperalgesia, which was demonstrated in our previous papers [6,24–26]. The difference in response of the ipsilateral paw to von Frey filaments approximately 12–16 days after nerve injury in the CCI rats compared to the control rats was:  $0.8 \text{ g} \pm 0.03$  vs.  $26 \text{ g} \pm 0.01$ , respectively, whereas the differences in the paw withdrawal test were  $5.6 \text{ s} \pm 0.4$  vs.  $8 \text{ s} \pm 0.6$ , respectively. The behavioral tests were conducted at 12–16 days after injury when neuropathic pain symptoms, like allodynia and hyperalgesia have been constant and persistent.

### Drug administration

Amitriptyline hydrochloride [3-(10,11-dihydro-5H-dibenzo[a,d]cyclopenten-5-ylidene) propyldimethyloamine], doxepin hydrochloride [11-(3-dimethylaminopropylidene)-6,11-dihydrodibenz[b,e]oxepine hydrochloride] and venlafaxine hydrochloride [(+/-)-1-[2-(dimethylamino)-1-(4-methoxyphenyl) ethyl] cyclohexanol hydrochloride] were obtained from Sigma–Aldrich, (Poznań, Poland). Morphine hydrochloride was obtained from Polfa (Kutno, Poland). Drugs were administered to the rats by one of two methods: (1) intraplantar (*ipl*) injection of a volume of 20  $\mu\text{l}$  per each animal to the ipsilateral hind limb sole, or (2) subcutaneous (*sc*) injection of a volume of 4 ml/kg body weight into the skin fold on the neck.

The range of doses was chosen according to our previous study [19] after *ip* injection and according to preliminary experiments with *ipl* and *sc* antidepressant administration. Most of the drugs (amitriptyline hydrochloride, doxepin hydrochloride and morphine hydrochloride) were dissolved in the *aqua pro injection*, but venlafaxine hydrochloride was dissolved in 12% dimethyl sulfoxide (DMSO). Control animals were injected *ipl* with the same volume of the respective vehicle. The dosages of the drugs for *sc* injections were calculated according to the body weight of each animal and were tested in accordance with the same schedule as described below. After completion of the experiment, the animals were euthanized by  $\text{CO}_2$  asphyxiation.

### Behavioral tests

Behavioral tests were conducted on days 12–16 after injury. Eight animals per an experimental group were used, with each animal used for one treatment only. In control groups, the number of animals was higher (up to 16) because the control group was included in each experiment and the results were pooled. In behavioral experiments, the control group comprised vehicle-treated (*ipl* or *sc*) CCI animals.

### Tactile allodynia (von Frey test)

Allodynia was measured before and 15, 30 and 60 min after *ipl* drug administration in the CCI rats. A set of calibrated nylon von Frey filaments (Stoelting, Chicago, IL, USA) was used. Animals were placed in plastic cages with wire net floor 5 min prior to experimentation. Increasing filament strengths were applied sequentially to the midplantar surface of the hind paw. The intensity of mechanical stimulation was increased from 0.2 to 26 g in a graded manner using successive filaments with greater pressure until the hind paw was withdrawn as previously described [6,19,27]. To determine tactile allodynia in rats, the strength of the von Frey stimuli ranged from 0.5 to 26 g. The ipsilateral paw was tested in animals in 4 cages twice over 3 min intervals using von Frey filaments, and the mean values were calculated.

### Paw withdrawal test–Hargreaves' test

Thermal hyperalgesia was measured on days 12–16 after induction of CCI at times before and 20, 45 and 75 min after *ipl* drug administration in the CCI rats. The pain threshold to high temperatures was tested using an Analgesia Meter (Landing, NJ). CCI rats were placed into 4 individual plastic cages with a glass floor 5 min prior to experimentation. A noxious thermal stimulus was focused through the glass onto the plantar surface of a hind paw until the animal lifted the paw away [6,28]. The cut-off latency was 20 s. The ipsilateral paws of the animals in 4 cages were tested twice over 3 min intervals, and the mean values were calculated.

## Data analysis

The behavioral data are presented in grams (von Frey test) and seconds (paw withdrawal test) as the mean  $\pm$  SEM of eight to sixteen rats per group. Some groups are more numerous because controls groups were repeated and the data were pooled when there was no significant differences. Inter-group differences were analyzed by ANOVA Bonferroni's multiple comparison test ( $*p < 0.05$ ;  $**p < 0.01$ ;  $***p < 0.001$  compared to vehicle-treated CCI exposed rats;  $^{\circ}p < 0.05$ ;  $^{\circ\circ}p < 0.01$ ;  $^{\circ\circ\circ}p < 0.001$  compared to morphine-treated CCI exposed rats;  $^{\#}p < 0.05$ ;  $^{\#\#}p < 0.01$ ;  $^{\#\#\#}p < 0.001$  compared to antidepressant-treated CCI exposed rats).

## Results

### The effect of intraplantar administration of amitriptyline, doxepin and venlafaxine on mechanical allodynia as measured by the von Frey test

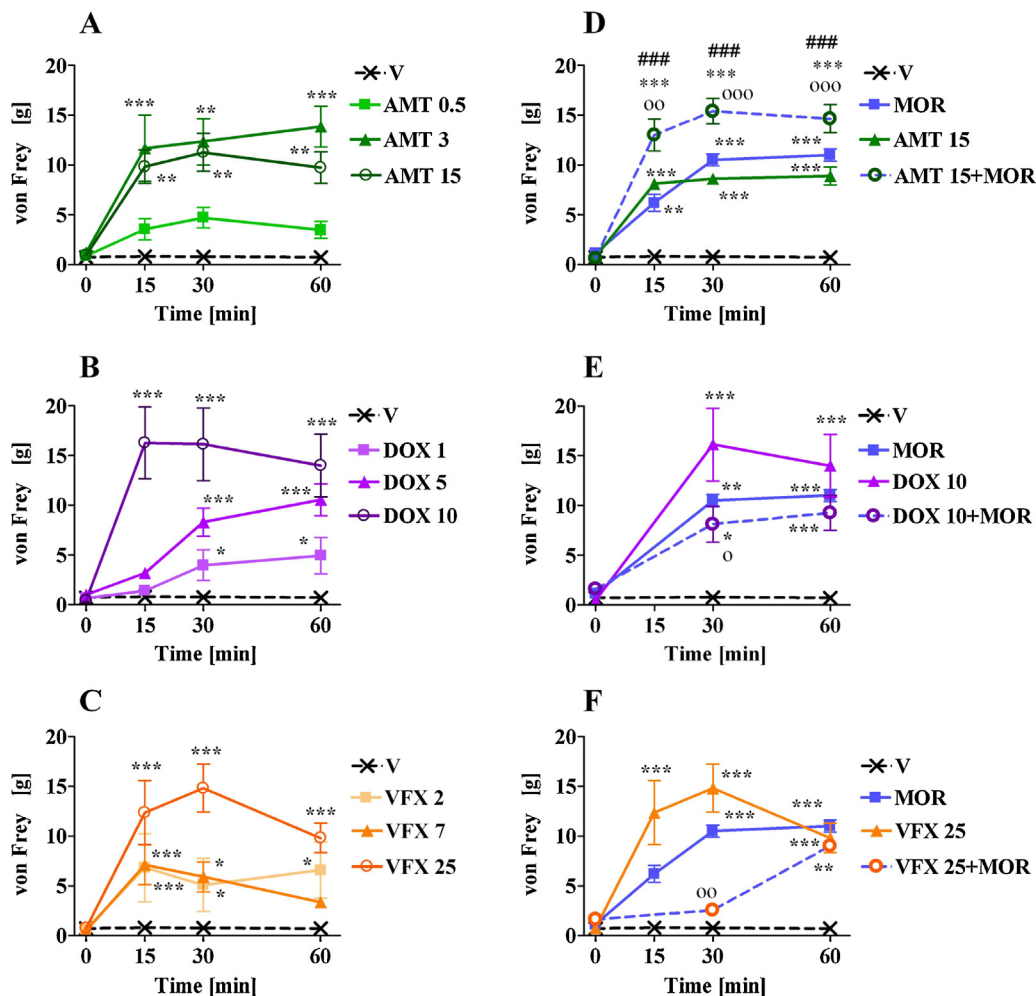
The lowest dose of amitriptyline (0.5 mg) had no significant antiallodynic action. Treatment with amitriptyline at doses of 3 and 15 mg showed a dose-dependent analgesic effect (Fig. 1A) by reducing allodynia at all the time points measured. This effect

remained significant from 15 min until 60 min after administration of these doses.

The lowest dose of doxepin (1 mg) showed a significant antiallodynic effect measured from 30 min up to 60 min after administration (Fig. 1B). Treatment with doxepin at a dose of 5 and 10 mg significantly reduced allodynia from 30 to 60 min or 15 to 60 min after administration, respectively. Treatment with venlafaxine at doses of 2 and 7 mg, significantly reduced allodynia at all of the time points measured or only in 15 and 30 min after administration, respectively. The dose of 25 mg had a significant analgesic effect at all time points measured (Fig. 1C).

### The effect of intraplantar co-administration of amitriptyline, doxepin and venlafaxine with morphine on mechanical allodynia as measured by the von Frey test

Amitriptyline at a dose of 15 mg exhibited a similar antiallodynic effect compared to morphine at a dose of 200  $\mu$ g as measured by the von Frey test (Fig. 1D). Co-administration of these two drugs significantly potentiated the antiallodynic effect vs. each drug alone at all time points measured. This effect was



**Fig. 1.** The effect of amitriptyline (AMT; 0.5, 3, and 15 mg; A), doxepin (DOX; 1, 5, and 10 mg; B) and venlafaxine (VFX; 2, 7, and 25 mg; C) injected to the ipsilateral hind limb sole (*ipl*) on mechanical allodynia as measured by von Frey filaments is presented in the left panel. The effect of *ipl* co-administration of amitriptyline (AMT; 15 mg; D), doxepin (DOX; 10 mg; E) or venlafaxine (VFX; 25 mg; F) with morphine (200  $\mu$ g) injected to the ipsilateral hind limb sole on mechanical allodynia as measured by von Frey filaments is presented in the right panel. The experiments were performed on days 12–16 after sciatic nerve ligation. The control group of CCI-subjected rats was treated with vehicle (V) instead of an antidepressant. The results are presented as the mean  $\pm$  SEM of pressure in grams. Inter-group differences were analyzed by ANOVA Bonferroni's multiple comparison test.  $*p < 0.05$ ;  $**p < 0.01$ ;  $***p < 0.001$  in comparison to vehicle-treated CCI-exposed rats;  $^{\circ}p < 0.05$ ;  $^{\circ\circ}p < 0.01$ ;  $^{\circ\circ\circ}p < 0.001$  compared to morphine-treated CCI-exposed rats;  $^{\#\#}p < 0.001$  compared to antidepressant-treated CCI-exposed rats.

statistically significant compared to the control group as well as to the groups treated with either amitriptyline or morphine alone.

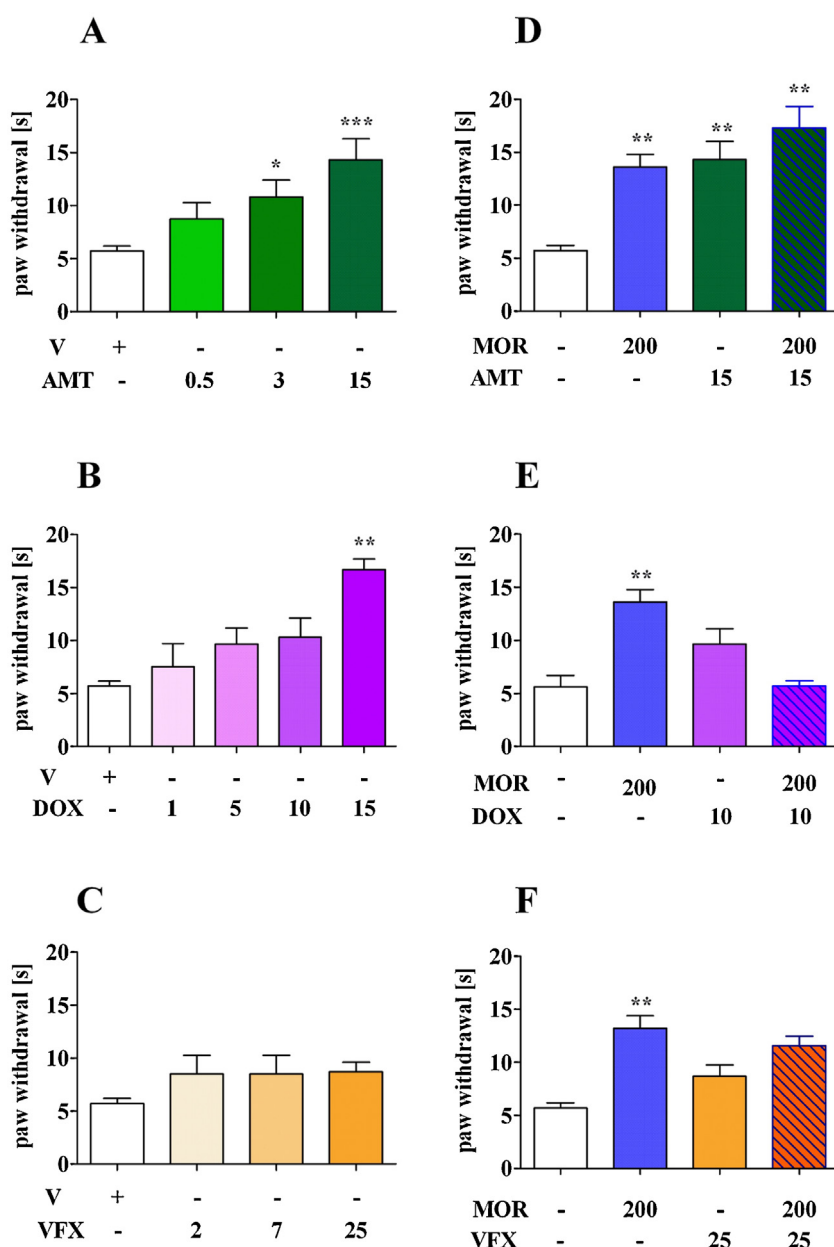
Doxepin at a dose of 10 mg exerted a strong antiallodynic effect similar to morphine at a dose of 200  $\mu$ g as measured by the von Frey test. Co-administration of doxepin with morphine produced a lower antiallodynic effect than each drug given alone (Fig. 1E).

Venlafaxine at a dose of 25 mg exerted a stronger antiallodynic effect compared to morphine at a dose of 200  $\mu$ g as measured by the von Frey test. Co-administration of venlafaxine with morphine elicited a lower antiallodynic effect than each drug given alone (Fig. 1F).

*The effect of intraplantar administration of amitriptyline, doxepin and venlafaxine on thermal hyperalgesia as measured by the paw withdrawal test*

Amitriptyline at a lower dose of 0.5 mg had no significant antihyperalgesic action. Treatment with amitriptyline at doses of 3 and 15 mg produced a dose-dependent significant antihyperalgesic effect at 45 (not shown) and 75 min or 20, 45 (not shown) and 75 min after administration, respectively (Fig. 2A).

The doxepin at doses of 1, 5 and 10 mg had no significant hyperalgesic effect. Treatment with doxepin at a dose of 15 mg significantly reduced hyperalgesia at 20 and 45 (not shown) and 75 min after administration (Fig. 2B).



**Fig. 2.** The effect of amitriptyline (AMT; 0.5, 3, and 15 mg; A), doxepin (DOX; 1, 5, 10, and 15 mg; B) and venlafaxine (VFX; 2, 7, and 25 mg; C) injected to the ipsilateral hind limb sole (*ipl*) on thermal hyperalgesia as measured by the paw withdrawal test is presented in the left panel. The effect of *ipl* co-administration of amitriptyline (AMT; 15 mg; D), doxepin (DOX; 10 mg; E) or venlafaxine (VFX; 25 mg; F) with morphine (MOR; 200  $\mu$ g) injected to the ipsilateral hind limb sole on thermal hyperalgesia as measured by the paw withdrawal test is presented in the right panel. The paw withdrawal test was conducted 20, 45 (not shown) and 75 min after drug administration, on days 12–16 after sciatic nerve ligation. The control group of CCI-subjected rats was treated with vehicle (V) instead of an antidepressant. The results are presented as the mean  $\pm$  SEM of time in seconds. Inter-group differences were analyzed by ANOVA Bonferroni's multiple comparison test. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  in comparison to vehicle-treated CCI-exposed rats.



**Table 1**

Comparison of intraplantar (*ipl*) and subcutaneous (*sc*) administration of amitriptyline (AMT, 15 mg), doxepin (DOX, 20 mg) and venlafaxine (VFX; 25 mg) in CCI-subjected rats. At days 12–16 after sciatic nerve ligation (CCI), antidepressants were administered either by *ipl* injection to the ipsilateral hind limb sole or by *sc* injection into the skin fold on the neck. Tactile allodynia (von Frey test) was measured 30 min after drug administration and thermal hyperalgesia (paw withdrawal test) was measured 45 min after drug administration. The control group of CCI-subjected rats was treated with vehicle instead of an antidepressant. The results are presented as the mean  $\pm$  SEM of pressure in grams and latency in seconds. Inter-group differences were analyzed by ANOVA Bonferroni's multiple comparison test.

CCI model	von Frey test (g)		Paw withdrawal test (s)	
	<i>ipl</i>	<i>sc</i>	<i>ipl</i>	<i>sc</i>
Vehicle	0.77 $\pm$ 0.06	0.72 $\pm$ 0.06	5.61 $\pm$ 0.28	5.69 $\pm$ 0.48
AMT (15 mg)	11.26 $\pm$ 1.90***	0.74 $\pm$ 0.13	11.25 $\pm$ 1.06*	6.92 $\pm$ 0.55
DOX (20 mg)	15.10 $\pm$ 3.63***	3.58 $\pm$ 0.58	20.25 $\pm$ 1.06***	5.21 $\pm$ 0.70
VFX (25 mg)	14.81 $\pm$ 2.40***	2.80 $\pm$ 0.50	8.75 $\pm$ 1.30	2.78 $\pm$ 0.48

\*  $p < 0.05$  compared to vehicle-treated CCI-exposed rats.

\*\*  $p < 0.01$  compared to vehicle-treated CCI-exposed rats.

\*\*\*  $p < 0.001$  compared to vehicle-treated CCI-exposed rats.

Venlafaxine at doses ranging from 2 to 25 mg had no effect on thermal hyperalgesia at all of the time points measured (Fig. 2C).

*The effect of intraplantar co-administration of amitriptyline, doxepin and venlafaxine with morphine on thermal hyperalgesia as measured by the paw withdrawal test*

Amitriptyline at a dose of 15 mg had a similar antihyperalgesic effect compared to morphine at a dose of 200  $\mu$ g as measured by the paw withdrawal test. Co-administration of amitriptyline with morphine slightly, nonsignificantly potentiated antihyperalgesic effect of each drug given alone (Fig. 2D).

Doxepin at a dose of 10 mg showed a nonsignificant antihyperalgesic effect. Morphine at a dose of 200  $\mu$ g caused a significant antihyperalgesic effect only at 75 min after administration. However, when the two drugs were co-administered, their effect was lower than that of each drug given alone at all time points measured (Fig. 2E).

Venlafaxine at a dose of 25 mg did not cause any significant antihyperalgesic effect at all time points measured. Morphine at a dose of 200  $\mu$ g caused a significant antihyperalgesic effect only at 75 min after administration. Co-administration of venlafaxine and morphine produced a similar effect as that observed after each drug given alone at all time points studied (Fig. 2F).

*Comparison of the effects of intraplantar and subcutaneous administration of amitriptyline, doxepin and venlafaxine on allodynia and hyperalgesia*

Administration of amitriptyline (15 mg) and doxepin (20 mg) had an antiallodynic and antihyperalgesic effect after *ipl* administration. However, no effect was observed after *sc* administration of either of these drugs. The effect of venlafaxine administration (25 mg) was significant in reducing tactile allodynia but not thermal hyperalgesia and was observed only after *ipl* administration (Table 1).

## Discussion

Antidepressants are drugs of choice for neuropathic pain treatment. They have recognized effects in some types of clinical syndromes. Since the first mention of imipramine as an analgesic in 1960 [29], they have been systematically used for neuropathic pain syndromes for 40 years [30]. The reduction of morphine effect in neuropathy after nerve injury could be attributed to the impairment of opioidergic transmission, e.g. due to a decreased number of presynaptic opioid receptors caused by loss of neurons [31–34]. Moreover, during long-term use of morphine its numerous side effects (e.g., respiratory depression, sedation, and constipation) and tolerance strongly limit its clinical use.

Therefore, lowering of the dose of morphine and its local administration with a drug which enhances its action would be beneficial during morphine treatment. Therefore, we investigated the analgesic effect of amitriptyline and doxepin (tricyclic antidepressants) as well as venlafaxine (a newer generation drug) alone and locally coadministered with morphine under neuropathic pain.

Our results show that when applied locally to the ipsilateral hind limb sole, all three antidepressants attenuated neuropathic pain symptoms which developed after sciatic nerve injury in rats. The intraplantar administration of amitriptyline (3, 15 mg), doxepin (1, 5, 10, 15 mg), or venlafaxine (2, 7 mg) was effective in antagonizing CCI-induced allodynia. Local mode of action was checked by a comparison with their subcutaneous injection at a site distant to the injured one. The antidepressants after *sc* administration did not induce alterations in pain thresholds, which mean that the effect of drugs was not a result of penetration to other tissues and in consequence was not caused by peripheral action. Local coadministration of amitriptyline with morphine significantly diminished allodynia and slightly hyperalgesia comparing with morphine alone. In contrast, coadministration of either doxepin or venlafaxine with morphine made it less effective in attenuation of neuropathic pain symptoms. The analgesic action of antidepressants after local administration deserves further study due to the first and only experimental and clinical data that focused on the analgesic efficacy of antidepressants after different methods of administration (oral, subcutaneous, intraperitoneal, intrathecal, topical) in different models of pain [9,15,35–38]. The analgesic effect of antidepressants is different from their action as a mood elevator. Their analgesic effect may occur soon after their administration, as much lower doses are needed compared to those used for the treatment of depression. The plasma concentration level of these drugs that is necessary to induce an analgesic effect is lower than that in a psychiatric practice. Investigations on the mechanism of action of antidepressants in pain treatment were tested in both animal and human models of acute and chronic pain. It was estimated that tricyclic antidepressants are effective in the model of neuropathic pain evoked by ligation of the sciatic nerve in rats [39]. Currently it is under consideration that antidepressant drugs act not only as mood elevators but also analgesics in chronic pain syndromes [40]. The antidepressant amitriptyline is used as an adjuvant in the treatment of a variety of chronic pain conditions. This drug interacts with many receptors and ion channels, including Na<sup>+</sup> channels. Antidepressants may also act by  $\alpha$ -adrenergic, histaminic and cholinergic postsynaptic receptors. These interactions play a role in their analgesic efficacy but are also responsible for common adverse effects, which can limit their use; thus, either topical or local administration may create more effective and safer therapy.

In our study, the dose-dependent action of amitriptyline and doxepin after local administration was documented. Allodynia attenuation after treatment with the highest dose of venlafaxine (25  $\mu$ g) was also observed. In the paw withdrawal test, the efficacy at the highest doses of amitriptyline and doxepin as well as a lack of activity after venlafaxine treatment was observed. Bomholt et al. [41] showed that in the formalin test, amitriptyline changed the ratio between different forms of pain behavior, i.e., increased second phase flinching behavior and reduced second phase licking behavior. In the chronic constriction injury model of neuropathic pain, amitriptyline fully reversed thermal hypersensitivity and had no influence on mechanical allodynia but significantly reduced mechanical hyperalgesia [41]. These responses are in accordance with our results, although a full reversal of thermal hyperalgesia in the paw withdrawal test was not observed. However, the influence of intraplantar administration on mechanical allodynia was important to address. Similarly, in an inflammatory model of pain, amitriptyline treatment reduced pain behavior in the mouse in the late phase after formalin administration [42], and this action was stronger than that of venlafaxine.

The effect of doxepin was similar to that of amitriptyline in our experiments. Its action against allodynia was dose-dependent. In contrast to both tricyclic antidepressants, venlafaxine did not reverse thermal hyperalgesia, but attenuation of allodynia was observed at all of the time points with statistical significance measured only at the highest dose (25 mg). This result is inconsistent with the results obtained in 2005 by Pedersen et al. [43] in which venlafaxine was ineffective against mechanical allodynia in CCI and spinal nerve ligation models. This study also reported that venlafaxine transiently increased the tail-flick latency in uninjured animals and attenuated second-phase flinching in the formalin test in rats [43]. Interestingly, Lang et al. [21] has shown that venlafaxine administered *per os* in the CCI model prevented the development of chronic pain if it was administered before the constriction to the sciatic nerve and reversed pain if it was given after, which is in agreement with our results.

In our investigation, all three antidepressants had an analgesic effect after topical use. Because there were no publications that directly compared these drugs, we focused on studies that topically administered these drugs in neuropathic pain models. Topically administered doxepin was effective, which is in accordance with the results from Gerner et al. [44]. The same results were observed after amitriptyline administration. Our results also confirm the observations by Sawynok et al., which topically administered amitriptyline in the formalin model in the rats [45]. Pain-related behavior induced by local administration of formalin is characterized by the occurrence of two phases of increased pain sensitivity in rats. In contrast, in neuropathic pain similar effects are observed only a short time after injury, but during development of neuropathy the inflammatory component is decreased. Therefore, inflammatory and neuropathic pain use the same mechanisms but differ in their presentation depending on time after inoculation or injury.

Currently, it is difficult to identify a single first choice drug for neuropathic pain treatment. It appears that coadministration of two or more drugs provides a better result with a lower risk of adverse events [46]. In neuropathic pain, the most commonly used drugs are narcotic analgesics, antidepressants, anticonvulsants, and some local anesthetics [47,48]. One of the important aspects in the treatment of neuropathic pain is combination therapy due to the participation of different mechanisms that may simultaneously interact with each other [49]. With a suitable combination of drugs, we are able to use submaximal doses with fewer adverse effects as well as to obtain more benefits from improved efficacy [50]. Coadministration of opioids with antidepressants has been suggested. In this group of drugs, tramadol is interesting because

its mechanism in the central nervous system is similar to that of antidepressants, i.e., by inhibition of monoamine reuptake from the synaptic cleft. Antidepressants inhibit the reuptake of serotonin and norepinephrine from synaptic cleft. These neuromodulators by themselves are stimulatory and are also the primary mediators in the descending antinociceptive pathways. The role of these neuromodulators is multidimensional, but something to consider is that activation of serotonergic and adrenergic pathways can induce antinociception. Inhibition of monoamine reuptake evokes pain transmission at the level of the dorsal horn in the spinal cord. In peripheral neuropathy, blocking alpha-adrenergic receptors may alleviate pain evoked by adrenergic stimulation of highly sensitive receptors at injured branched peripheral nerves [51,52]. The mechanisms of interaction of antidepressants with the opioid system remain unclear. In 1998, Gray et al. [53] showed that when acetorphan, an inhibitor of enzymatic enkephalin degradation, is given before administration of an antidepressant, the analgesic action of morphine and antidepressant activities are intensified. It is considered that in this case, endogenous peptide release occurs in the region of opioid receptors after systemic (intraperitoneal) administration of antidepressants. There are other investigations that have focused on the increase of endogenous opioids in the striatum and nucleus accumbens of rats after chronic administration of antidepressants [54]. A question regarding the involvement of the opioid system after local administration of antidepressants still remains unclear. Initial investigations suggested that tricyclic antidepressants have a low affinity to opioid receptors [55]. Venlafaxine has been characterized as being able to act on the opioid system and enhance opioid analgesia when administered with a selective opioid agonist, and this action is inhibited by selective opioid antagonists. [56]. Potentiation of hyperalgesia may be related to the reported influence of venlafaxine on kappa opioid receptors because its effect in mice was blocked by norBNI, a kappa opioid receptor antagonist [56]. It was documented that in neuropathic pain, the increased activity of the prodynorphin system is responsible for the mechanism of allodynia [27]; therefore, the mechanism of action of venlafaxine may be responsible for the attenuation of the morphine effect.

## Conclusions

Our results suggest that under neuropathic pain in the rat, in contrast to amitriptyline, which potentiated morphine analgesia, venlafaxine and doxepin attenuated its effect, although further studies are necessary to precisely describe the mechanism of this interaction. Our results suggest that the use of amitriptyline with morphine may be beneficial due to their interactions. Experimental characterization of also other antidepressants that could be proposed for local coadministration with opioid drugs in neuropathic pain could improve therapy in the future.

## Conflict of interest

The co-authors of this paper declare that they have no conflict of interest.

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